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Journal of Colloid and Interface Science 300 (2006) 117–122
JOURNAL OF Colloid and Interface Science
www.elsevier.com/locate/jcis

Facile fabrication of poly(*p*-phenylene ethynylene)/colloidal silica composite for nucleic acid detection

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Received 13 January 2006; accepted 27 March 2006
Available online 3 April 2006

Abstract

Fabrication, characterization, and application of poly(phenylene ethynylene) (PPE)/silica composite particles are described. PPE is a class of conjugated polymers, which has been used for various sensory materials. However, its hydrophobic nature makes its application difficult in the aqueous phase, especially for biological substance detection. In this report, we utilized non-aqueous soluble PPE, 15 nm of colloidal silica particles, and aminosilane to fabricate a biosensory platform. The resulting composite showed high aqueous compatibility, large surface area, high quantum efficiency, and versatile chemical modification including oligonucleotide coupling. By monitoring the fluorescence quenching of PPE, we could detect a quencher-labeled target oligonucleotide specifically. Stern–Volmer (SV) analysis showed different accessibility of fluorophores (PPE) to a quencher labeled target oligonucleotide. The accessibility of fluorophores and SV constant are determined to be 0.54 and 4.2×10^7 M^{-1}, respectively, from a modified SV plot. This method will broaden the capability of conjugated polymers for the sensitive detection of biological substances.

Keywords: Poly(phenylene ethynylene); Nanoparticle; Fluorescence quenching; Stern–Volmer analysis; Nucleic acid detection

1. Introduction

Conjugated polymers (CPs) have been used extensively for the sensitive detection of various analytes such as chemicals, metals, and biological substances using fluorescence quenching or fluorescence resonant energy transfer (FRET) [1–5]. The high sensitivity of CPs can be attributed to signal amplification originating from the collective response of molecularly wired fluorophores to analytes [6]. Despite the success of CPs, the hydrophobicity of most CPs complicates their application to the detection of biological substances in aqueous media. By introducing hydrophilic side chains on CPs, researchers demonstrated the detection of oligonucleotides [7] and proteins [8] in water. However, incorporation of charged groups onto a backbone of CPs generally results in a significant loss of luminescence quantum efficiency and increased susceptibility to environmental factors such as pH, temperature, salinity, and surfactant [9]. Therefore, we wished to develop a CP-based sensory system showing improved compatibility with aqueous media, while retaining the synthetic versatility and relatively high quantum efficiency of organic-soluble CPs.

Here we report a facile fabrication method for a composite of poly(phenylene ethynylene) (PPE) on colloidal silica useful for the detection of oligonucleotides in aqueous media. The composites were constructed by mixing **P1**, colloidal silica, and an aminosilane in an organic solvent, followed by precipitation in water. The product was silica particles with an ultrathin coating of **P1** and amine functional groups on the surface. The composite retained the luminescence quantum yield of the corresponding **P1** and has a high surface area. The robustness of the composite allowed for chemical modification in both organic and aqueous solvents. Owing to the hydrophilicity and high specific gravity of silica, covalent attachment of probe oligonucleotides and purification is performed in aqueous media by applying a simple process of centrifugation and decantation. Finally, we demonstrate fluorescence quenching of the probe oligonucleotide grafted composite by specific hybridization of a quencher labeled target oligonucleotide. Details on quencher accessibility to the fluorophores in the complex were also quantified by modified Stern–Volmer analysis.

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0021-9797/$ – see front matter
doi:10.1016/j.jcis.2006.03.063

2. Materials and methods

2.1. General

Chemicals, including solvents, were purchased from Aldrich and used as received. Palladium(0) catalyst was purchased from Strem, and (3-trimethoxysilylpropyl)diethylenetriamine was purchased from Gelest. 20 wt% of colloidal silica dispersed in dimethylacetamide (DMAC) (DMAC-ST) was provided by Nissan Chemical. The mean particle size provided by the manufacturer was 10–15 nm. 3-Maleimidopropionic acid *N*-hydroxysuccinamide (MPS) was purchased from Molecular Biosciences, Inc. 2XHybridization solution was purchased from Sigma, and aliquots were made by dilution with water (1X). The aliquots were stored at −20 °C until used for hybridization. All oligonucleotides were purchased from Biosource International (CA), and aliquots were made as they were received. The typical concentration of the aliquots was 10^{-5} M. 5′-SH-CGAGCCTTTACACCGACTCCAACAGCT CG3′ (probe), 5′-AGCTGTTGGAGTCGGTGTAAAGGCTC-Cy5-3′ (true target), and 5′-GTC TCA GAC AGT TGT CG-Cy5-3′ (non-target). Purification of the thiol-modified oligonucleotide was carried out by using a NAP5 column according to the manufacturer's protocol. UV–vis spectra were recorded using a Hewlett–Packard 8453 Diode array spectrophotometer. Fluorescence spectra were obtained using a FluoroMax. The molecular weight of the polymer was determined by gel-permeable chromatography (GPC) using a Plgel 5 μm MiniMIX-C column (Polysciences, Inc.) in 0.1 M LiBr in DMF. The molecular weight is reported relative to PMMA standards purchased from Polysciences, Inc. A transmission electron microscopic (TEM) study was carried out using a JEOL JEM 100 CX scanning transmission electron microscope. TEM samples were prepared by placing a drop of the particle solution on one side of a Cu grid. After a few minutes, excess solution was removed by wicking it away with filter paper. Elemental analysis was carried out by Desert Analytics (Tucson, AZ). A Brunauer–Emmet–Teller (BET) isothermal gas adsorption experiment was performed using an ASAP 2020 accelerated surface area and porosimetry analyzer (Micromeritics, Inc.) under an Ar atmosphere. Particle size was measured by light scattering using a Horiba laser scattering particle size distribution analyzer (LA-910).

2.2. Synthesis

P1 was synthesized by a Pd(0)-catalyzed cross-coupling of the pentiptycene diacetylene (**2**, 1.22 g, 2.55 mmol) and **3** (1.92 g, 2.50 mmol) in a degassed solution of DMF/morpholine (6/4 = v/v) at 80 °C for 16 h. The polymer was recovered by precipitation in methanol, redissolved, and precipitated in acetone to obtain a yellow powder (1.4 g, yield 56%). The molecular weight (M_n) and polydispersity index (PDI) were estimated by GPC to be 28,000 and 1.6, respectively (PMMA standards in 0.1 M LiBr DMF). ^{1}H NMR (400 MHz, DMSO-d6): δ 8.47, 8.23, 7.86, 7.72, 7.24, 7.10, 6.84, 5.40, 5.25, 4.16, 4.64, 3.55, 2.37, 2.25, 2.02.

Monomer **3**: 18.8 g (31.9 mmol) of **4**, 9.3 g (76.4 mmol) of 2-amino-2-(hydroxymethyl)-1,3-propanediol, and an excess (∼20 g) of K_2CO_3 were combined with 300 ml of DMSO. The reaction mixture was stirred overnight at room temperature. After filtering off the K_2CO_3, the filtrate (DMSO) was removed by a vacuum distillation. Excess water was added to the resulting viscous oil, resulting in a white powder precipitation. The precipitates were collected by filtration and dried under vacuum. 20.3 g (26.4 mmol, 83%) of white powder was collected. ^{1}H NMR (400 MHz, DMSO-d6): δ 7.32 (s, –N(CO)H–, 2H), 7.13 (s, ArH, 2H), 4.76 (t, $–CH_2OH$, 6H), 3.94 (t, Ar–OCH_2–, 4H), 3.52 (d, $–CH_2OH$, 12H), 2.23 (t, $–CH_2C(O)NH–$, 4H), 1.68 (m, $–CH_2CH_2–$, 8H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.60, 152.33, 122.39, 86.95, 69.29, 62.28, 60.73, 35.31, 28.15, 21.98. Exact mass ([M + H]): calcd for $C_{24}H_{38}I_2N_2O_{10}$, 769.0689; found, 769.0688.

2.3. Fabrication of *P1*/silica composite

0.2 ml of 20 wt% colloidal silica sol in DMAC, 2 ml of DMAC, and 0.1 ml of NH_4OH were combined and stirred rapidly for 30 min. 2.2 ml of **P1** dissolved in DMAC (1 mg/ml) was transferred into the silica solution, followed by the addition of 0.1 ml of 3-(trimethoxysilylpropyl)ethylenetriamine. The solution became slightly viscous after 30 min of stirring at room temperature, and then the solution was transferred into 6 ml of water with rapid stirring. Precipitates were collected as a pallet from centrifugation and decantation, and the pallet was resuspended into DMAC. The composite in DMAC was centrifuged again, and yellowish supernatant was discarded. Various organic solvents including DMF, DMSO, and *N*-methyl 2-pyrrolidinone (NMP) were used to further wash off unreacted PPE from the composite. This washing step was repeated (usually two to three times), until no color was observed from the supernatant. The composite particle was then washed with water three times and stored in water for the oligonucleotide coupling. The composite was lyophilized for quantification, resulting in an off-yellow powder (48 mg).

2.4. Activation of the composite and probe oligonucleotide coupling

For the efficient coupling chemistry of MPS with amine, we changed the solvent from water to DMF by centrifuging/decanting (three times). 1 ml of the composite in DMF and ∼10 mg of MPS were combined in a 1.5 ml centrifugal tube, and agitated in a Fisher Minishaker set at 1400 rpm for 1.5 h. Excess MPS was removed by rinsing with 1 ml of fresh DMF five times, and the supernatant was discarded. The maleimide-activated composite particle in ∼0.03 ml of residual DMF was transferred into a 0.9 ml of desalted oligonucleotide solution. The oligonucleotide was desalted using a NAP5 column as directed by the manufacturer's manual. The solution was placed in the shaker overnight. The probe-attached composite was thoroughly cleaned by centrifugation with 1 ml of 0.1% SDS (three times) and decantation with fresh water (three